AGRICULTURAL EXPERIMENT STATION

University of Puerto Rico — Río Piedras, Puerto Rico

JAIME BENÍTEZ, M.A., LL.M. ..... *Chancellor*
LUIS STEFANI, M.S. ..... *Vice Chancellor*

STATION STAFF [1]

ARTURO ROQUE, M.S. ..... *Director*
B. G. CAPÓ, Ph.D. ..... *Asst. Dir. for Research*
R. MOLINARY-SALÉS, B.S. ..... *Editor*

Agronomy and Horticulture:
_____, Head of Dept.
P. Arcamuzi, M.S., Assoc. Agron.
P. Landrau, M.S., Asst. Agron.
F. Méndez, M.S., Assoc. Agron.
W. Peacock, M.S., Assoc. Hort.
J. P. Rodríguez, B.S., Assoc. Agron.
M. A. Valencia, B.S., Res. Asst. in Soil.
F. Vázquez, B.S., Res. Asst. in Soil.

Animal Husbandry:
_____, An. Husb., Head of Dept.
L. Rivera Brenes, M.S., Assoc. An. Husb., Acting Head of Dept.
J. L. Cabrera, M.S., Asst. An. Husb.
L. P. Calzo, B.S., Res. Asst. in Chem.
E. del Toro, Jr., M.S., An. Husb.
F. J. Marbán, M.S., Asst. Chem.
J. Martínez, B.S., Res. Asst. in Bact.
J. Rivera-Anaya, D.V.M., Assoc. Vet.

Chemistry:
_____, Head of Dept.
A. García [2] B.S., Asst. Chem.
L. Igaravidez, B.S., Asst. Chem.
B. López-Romero, B.S., Res. Asst. in Chem.
I. Rickenbach, M.S., Asst. Chem. (Mrs.)
F. Sánchez-Nieves, M.S., Assoc. Chem.

Economics:
M. Hernández,[2] Ph.D., Econ., Head of Dept.
I. Avilés,[2] M.S., Res. Asst. in Econ.
H. Bayrón, B.S., Res. Asst. in Econ.
A. Berges,[2] B.S., Res. Asst. in Rural Soc.
J. R. Calderón, B.S., Res. Asst. in Econ.
G. Espinet, B.S., Res. Asst. in Econ.
G. Gastón,[2] B.S., Res. Asst. Econ.
D. Haddock, M.S., Assoc. Econ.
L. Hernández, B.S., Res. Asst. in Econ.
M. Pérez,[2] B.S., Asst. Econ.
F. Román,[2] B.S., Asst. in Econ.
G. Sosa, Ph.D., Econ., Acting Head of Dept.
E. Silva,[2] B.E., Res. Asst. in Econ.
P. Vázquez-Calzada,[2] M.S., Assoc. Econ.

Entomology:
G. N. Wolcott, Ph.D., Ent., Head of Dept.
L. F. Martorell, Ph.D., Assoc. Ent.
M. Pérez, M.S., Asst. Ent.

Plant Breeding:
_____, Plant Breeder, Head of Dept.
P. Gandía-Díaz, M.S., Assoc. Plant Breeder, Acting Head of Dept.
B. Becerra, M.S., Res. Asst. in Plant Brdg.
F. Marrero-Tríay, M.S., Asst. Plant Breeder.
C. Mascaró,[2] B.S., Res. Asst. in Plant Brdg.
J. Vélez-Fortuño, M.S., Asst. Plant Breeder.

Plant Pathology:
I. A. Alvarez, M.S., Plant Path., Head of Dept.
J. Abreu, B.S., Plant Path.
J. Bird,[2] B.S., Res. Asst. in Plant Path.
A. Cruz-Miret, B.S., Res. Asst. in Plant Path.

Plant Physiology:
_____, Plant Physiol., Head of Dept.
M. del C. C. Fernández, Ph. C., Asst. Plant Physiol., (Mrs.)
E. Hernández, M.S., Asst. Plant Physiol.
J. Martínez, B.S., Res. Asst. in Chem.
G. Samuels, Ph.D., Visiting Plant Physiol.
M. A. Tío, M.S., Asst. Plant Physiol.

Soils:
J. A. Bonnet, Ph.D., Soil Chem., Head of Dept.
M. A. Lugo-López, Ph.D., Assoc. Soil Sci.
G. Nieves Cuevas, B.S., Res. Asst. in Chem.
A. Rivera, B.S., Asst. Chem.
J. Roldán, B.A., Res. Asst. in Chem.

School of Tropical Medicine:
D. Fernández, B.S., Res. Asst. in Chem. (Misc)
A. Muñiz, B.S., Res. Asst. in Chem. (Misc)
G. M. V. de Urrutia, B.S., Res. Asst. in Hum. (Misc.)

Institute of Nutrition:
L. D. Rodríguez, M.S., Res. Asst. in Chem. (Misc)
R. Suárez, B.S., Res. Asst. in Chem.

Library:
M. L. Benmaor, M.A., Asst. Libr. (Misc)

Service Departments:
C. J. Clavell, B.S., Admin. Asst.
R. Gross, B.S., Res. Asst. in Agron. in Charge of Station Farm

Río Piedras Plants:
V. Rodríguez-Suárez, M.Sc. Tech. Dir.
H. Bou, M.S., Asst. Chem.
E. de Bruguera, B.S., Res. Asst. in Chem.
V. Pesquí de Landrau, M.S., Asst. Chem. (Mrs.)
I. Rivera, M.S., Asst. Chem. (Misc)

Land Farm Main Office:
E. Vincenteño, B.S., Agron. Head of Div.
G. J. Rigau,[2] B.S., Res. Asst. in Agron.

Isabela Substation:
A. Ridkam, M.S., Agron. in Charge
C. M. Cabrera, B.S., Res. Asst. in Agron.
Frank L. Mojer, B.S., Res. Asst. in Agron.

Isabela Seed Farm:
M. Avilés, M.S., Asst. Agron. in Charge
M. Guevara, M.S., Asst. Agron.

Juana Farm:
A. González-Chapel, M.S., Assoc. An. Husb. in Charge
P. Gabel, B.S., Res. Asst. in An. Husb.
L. B. Ortiz, M.S., Res. Asst. in Agron.
C. Ortiz-Lugo, B.S., Asst. Agron.

Corozal Farm:
H. Gandía-Díaz, B.S., Assoc. Agron. in Charge
B. Olivencia, M.S., Res. Asst. in Agron.
C. Rivera-López, B.S., Asst. Agron.

Coffee Farm:
A. Rodríguez-Cabrera, M.S., Asst. Agron. in Charge
S. Rodríguez, B.S., Res. Asst. in Agron.

Tobacco Farm:
A. S. Amy, B.S., Assoc. Agron. in Charge

La Plata Animal Prod. Substation:
M. Rojo, M.S., Asst. An. Husb.
L. Cadilla, B.S., Res. Asst. in An. Husb.

Textile Asts Plant Propagation Substation:
J. L. Blanes, B.S., Asst. Agron. in Charge

---

[1] As of date of issue—June 30/70
[2] Leave on partial studies
[3] Other leave

THE JOURNAL OF AGRICULTURE
OF THE UNIVERSITY OF PUERTO RICO

Issued quarterly by the Agricultural Experiment Station of the University of Puerto Rico, for the publication of articles by members of its personnel, or others, dealing with any of the more technical aspects of scientific agriculture in Puerto Rico or the Caribbean Area.

VOL. XXXI JULY 1947 No. 3

TABLE OF CONTENTS

A QUINTESSENCE OF SENSITIVITY: THE COFFEE LEAF-MINER — PAGE
by George N. Wolcott 215

THE LEAFHOPPERS OF WHITE CLOVER, *Trifolium repens* L. IN NORTHERN NEW YORK AND IN THE MOUNTAINS OF HAITÍ
by George N. Wolcott 220

BENZENE HEXACHLORIDE AS A TERMITE REPELLENT
by George N. Wolcott 224

THE SYNONOMY OF *Empoasca fabalis* DELONG
by John S. Caldwell 226

A STUDY OF THE EFFECT OF SIX QUALITATIVE CHARACTER PAIRS ON YIELD AND COMPONENT CHARACTERS IN THE F_2 OF A CROSS BETWEEN TWO VARIETIES OF BARLEY
by F. Mariota-Trías 227

STUDIES ON VIRUS DISEASES OF PAPAYA (CARICA PAPAYA) IN PUERTO RICO

I. TRANSMISSION OF PAPAYA MOSAIC
by José Adsuar 248

II. TRANSMISSION OF PAPAYA MOSAIC BY THE GREEN CITRUS APHID (*Aphis Spiraecola Patch*)
by José Adsuar 257

III. PROPERTY STUDIES OF PAPAYA MOSAIC VIRUS
by José Adsuar 260

Published By
UNIVERSITY OF PUERTO RICO
THE AGRICULTURAL EXPERIMENT STATION
Rio Piedras, P. R.
Issued, June 1950

General Supplies Administration
Service Office – Printing Division
SAN JUAN, P. R.
1950

A QUINTESSENCE OF SENSITIVITY: THE COFFEE LEAF-MINER

By GEORGE N. WOLCOTT [1]

High humidity, with many rainy days, characterizes the climate of the lower mountainous slopes of tropical America where Arabian coffee, *Coffea arabica* L., grows. Even on sunny days, clouds often drift by overhead, their passing shadows inducing the sudden darting flight of numerous little satiny white moths, the adults of the coffee leaf-miner, *Leucoptera coffeella* Guérin-Méneville. When the sun reappears, flight ceases as suddenly as it began, and the coffee grove that was alive with the flashes of their wings a minute ago now appears deserted of active insect life.

Despite their small size, they are quite conspicuous in flight, but when at rest they closely fold their silvery wings around their body. The sudden, positive manner in which these minute insects react to a variation in light intensity is characteristic of their reactions to other natural factors of their environment.

Humidity is decisive in determining in what parts of the grove heavy leaf-miner infestations will develop, altho to temperatures within the limits at which coffee thrives, the insects appear relatively insensitive. The female moths tend to oviposit mostly on wind-swept ridges where the evaporating power of the wind is greatest, and least at the bottom of ravines where the wind can not penetrate, and relative humidity remains high at all times. Climbing the slope, one finds all gradations in the amount of infestation, with the maximum always where the action of the wind is most marked. Porous-cup atmometers show this in the coffee grove, as does artificial rearing of the insect in separate compartments of a greenhouse of which the humidity can be controlled. There is the very best of reasons why the female moths should prefer the more exposed plants for oviposition, as during prolonged periods of rain the mines of the caterpillars in the coffee leaves become filled with water, in which the caterpillars drown. During such weather, it is

[1] Head, Department of Entomology of the Agricultural Experiment Station, University of Puerto Rico.

in the exposed situations that the wind dries out the leaves most rapidly and it is here that the insect will have the best chance for survival. Indeed, it is on the single factor of humidity that most of the observed differences in abundance of leaf-miner depend.

The scarcity of the leaf-miner in Haiti, as compared with its abundance in Puerto Rico, is due to local methods of no cultivation, and allowing coffee trees to grow wherever volunteer plants had happened to sprout, making such a dense tangle of trees that humidity is always high, while soaring mountains often cut off all exposure to winds. New plantings in Haiti, with small trees widely spaced and open to the wind, invariably suffer heavy infestation just as they do in Puerto Rico. The scarcity approaching total absence of coffee leaf-miner in old Haitian groves is definitely not due to some new and more efficient larval parasite, for all collected in Haiti had previously been known from Puerto Rico, and *Chrysocharis lividus* Ashmead, reported by O. W. Barrett as long ago as 1905 and often the most abundant of any in Puerto Rico, does not occur in Haiti at all. Even proper wide spacing of the trees fails to provide a suitable environment for the leaf-miner in Trinidad, which is out of the trade wind and hurricane belt, and its coffee groves have maximum humidity in the ravines in the Northern Range of mountains.

Sensitive as the ovipositing female moth is to differences in humidity, apparently she does not distinguish between Arabian coffee with tender leaves suitable for the development of her young, and such a tough-leaved species as *Coffea stenophylla* Don. The minute caterpillar developing within a leaf-miner egg laid on such a tough leaf hatches normally, and without emerging from the protective covering of its egg-shell, eats thru it directly into the leaf tissue beneath. It attacks the tough leaf tissue with apparent success at first, but often by the second day all activity has ceased, and the little caterpillar is dead. Had the egg been laid on a leaf of Arabian coffee, the minute caterpillar would normally continue eating a cavity into the central tissue of the leaf until this is large enough so that it could crawl entirely out of the egg-shell. Like most kinds of insect larvae living in a protected situation, its flattened body is opalescent whitish, the head somewhat darker, with the sharp edges of its jaws almost black. It continues to eat and increase in size within its mine in the coffee leaf, feeding only

on the most tender cells and avoiding those of both the upper and lower outside skins of the leaf.

Leaf-miner larvae attacking some other kinds of leaves are sometimes precipitated into the outer world during rainy weather, when the thin outer skin of the leaf they have attacked is rotted by rain. Those of even the most tender coffee leaf are of tougher texture and resist decomposition at least long enough for the caterpillar to complete its growth, but unfortunately are sufficiently permeable to rain-water so that it collects in the mine, distending it almost to bursting.

Drowning in its rain-water filled mine is not the only peril besetting the coffee leaf-miner caterpillar in its mine. Numerous kinds of minute parasitic wasps occur in coffee groves, which, with their minute sharp ovipositor, may puncture the skin of the mine and lay their egg on or within the body of the caterpillar. In Puerto Rico, Mr. Francisco Seín has reared ten kinds of these minute wasps which are either parasites or super-parasites on the coffee leaf-miner. Arranged approximately in the order of their abundance, their names, as determined by Mr. A. B. Gahan of the U. S. National Museum, are as follows:

Closterocerus leucopus Ashmead
Chrysocharis lividus Ashmead
Horismenus cupreus Ashmead
Zagrammosoma sp. nov.
Closterocerus sp. near *cinctipennis* Ashmead
Cirrispiloideus sp. nov.
Derastenus sp. near *fullawayi* Crawford
Tetrastichus sp. nov.
Telenomus sp.
Microbracon sp. (det. C. F. W. Muesebeck)

In coffee groves near the coast and at no great elevation, some of these minute leaf-miner parasites are at times reasonably abundant, but at the higher elevations where most coffee is produced, they are far from numerous and usually are so scarce that all of them together rarely destroy more than an eighth or a tenth of all leaf-miner caterpillars. From the commercial standpoint, therefore, they are of negligible importance, and even the entomologist finds them so scarce that careful studies of each one, to determine its status and non-parasitic habits, are almost impossible. This list of parasites is longer from Puerto Rico than from the other Larger Antilles

only because studies here have been more intensive, but the same or similar parasites occur in the coffee groves of all of these larger islands.

Quite different is the parasitic situation in the coffee-producing islands of the Lesser Antilles, for Mr. Seín discovered a previously undescribed parasite, later named *Mirax insularis* by C. F. W. Muesebeck, to be normally responsible for the destruction of sixty to eighty-five percent of all leaf-miner caterpillars in the island of Guadeloupe, F. W. I., and only slightly less effective in other adjacent islands. Introduced into Puerto Rico in 1937, it is unquestionably established in the mountains around Lares, but even when present in greatest abundance, attacks less than three percent of the leaf-miner caterpillars, and at most times only a small fraction of one percent will be found parasitized by *Mirax*. The desirability is obvious of the most careful comparative ecological study of the requirements of each and every parasite of the coffee leaf-miner in every island of the West Indies. The establishment in coffee groves of the optimum conditions for the development of some or most of these parasites may be impossible of practical attainment, but the factor lacking for one or several may be readily supplied, if we know what that factor is, and thus result in commercial leaf-miner control.

When fully-grown, the leaf-miner caterpillar is less than a quarter of an inch in length, smaller indeed than many other kinds of caterpillars just hatched from the egg. It acts like some of them also, in a most interesting way, for emerging from its mine when the wind is not blowing, it spins a long thread of silk with incredible swiftness as soon as a breeze begins to blow. If the breeze stiffens, the thread soon becomes so long as to be buoyant to the insignificant weight of the caterpillar, so that as soon as the thread breaks loose from attachment, the caterpillar may be carried far from the leaf from which it obtained its nourishment. This phenomenon can be most readily observed during a period of fitful breezes: the dropping of the caterpillars on their threads as soon as the breeze starts, and sudden halt when the breeze stops, followed by a prompt lengthening of the threads again as the breeze starts. If the breeze stops entirely, the caterpillar is left indefinitely suspended in mid-air, a ready prey to any passing reinita or warbler.

Spinning of the cocoons may take place on any leaf, not necessarily of coffee, but of any plant that happens to be present in the grove. In the selection of a position for the cocoon, the caterpillar is sensitive to varying intensities of light, for in complete shade the cocoon may be formed on the upperside of a leaf. Normally, however, it is on the underside, the caterpillar first constructing a silken hammock under a slightly raised portion of the leaf, this support of white silk often taking the form of an X. Upon this silken mattress the caterpillar spins the cocoon proper, within which it transforms to pupa. It remains as pupa three to nine days. The moth leaves the cocoon at night, mates, and often has completed oviposition by the next day.

Within the short span of its life-history, the leaf-miner both as adult and as caterpillar has shown itself very sensitive to variations in light intensity; as adult to humidity, and as caterpillar to air in motion. When as much is known about the reactions of each of the parasites of the leaf-miner to natural conditions in coffee groves as is known of their host, we may anticipate much more efficient natural control of this pest of coffee by its parasites.

January 8, 1943.

Footnote:

This summary of observations on the coffee leaf-miner made with and by Mr. Francisco Seín Jr., is published at the present time because of his departure from the Experiment Station, effective June 30, 1948, after continuous employment there since October 8, 1918. The original publication of most of these observations is scattered thru the reports of the Division of Entomology in the Annual Reports of the Experiment Station from shortly after that date almost up to the present.

GEORGE N. WOLCOTT.

THE LEAFHOPPERS OF WHITE CLOVER, *Trifolium repens* L., IN NORTHERN NEW YORK AND IN THE MOUNTAINS OF HAITI

By GEORGE N. WOLCOTT [1]

A bountiful and never-failing spring of clear, sparkling water flowing out of the mountainside is largely responsible for the intensive agricultural development of the region around Kenscoff, Haiti. The abundance of water makes possible the production of such crops as watercress just below the springs, and of lettuce, onions, cabbage, potatoes, carrots and other temperate zone vegetables by irrigation at somewhat lower levels, and more especially of artichokes. Peach trees thrive at an elevation of around 4,000 feet, now producing an abundance of fruit since the apparently perfect natural control of the West Indian peach scale, *Pseudalacaspis pentagona* (Targioni), resulting from the introduction of the twice-stabbed ladybeetle, *Chilocorus cacti* L. The effectiveness of this ladybeetle, endemic in Texas and Cuba, whence it was brought to Puerto Rico and then sent to Hispaniola, is seriously lessened at lower elevations in the tropics by the abundance of predaceous lizards, but these are notably scarce so high in the mountains. Indeed, both flora and fauna are primarily temperate zone, the original forest being replaced by the first European settlers with the plants with which they were familiar in France, not only fruits and vegetables, but also the same grasses and clovers, and not excepting the weeds. The pastures of the steeper slopes not in irrigation to a large extent are composed of white clover, furnishing a rich forage for cows whose milk is the basis for a small butter and cheese industry.

Sweeping areas in white clover (*Trifolium repens* L.) mixed with European grasses in a field where sweet-peas had been grown for export last year, the largest, most abundant and by far the most conspicuous leafhopper present on the clover on February 1, 1948, was found to be *Hortensia similis* (Walker). Originally described from Central America as a *Tettigonia*, and for many years called a *Kolla*, this common neotropical leaf-

[1] Head, Department of Entomology of the Agricultural Experiment Station, University of Puerto Rico.

hopper, opaquely light green above, with a distinctive and unvarying pattern in black on head and pronotum, was possibly most intensively studied at the time when mosaic disease of sugar-cane was invading Puerto Rico, for it seemed to be the most probable vector of the disease.(1) In fields of young cane, its abundance varies directly with rainfall. During drought, the adults retreat to moist meadows of "malojillo" or Pará grass (*Eriochloa subglabra* and *Panicum barbinode*), to stream margins, or to the grasses of mountain pastures and the margins of forest and coffee groves. Nymphs have been reared on young sugar-cane plants, and occur in abundance on grasses, but rarely are found on corn, beans, carrots and weeds, from which adults have been swept. The nymphs "feed nearly half the time. With their thick beak inserted in the cane plant, they let go with their legs, using them to get rid of the minute drop of colorless excreta which collects at the anus. With all their legs in motion at once and these little drops of moisture being hurled into the air at the rate of one every seven seconds, a colony of *Kolla similis* nymphs feeding is a most exciting spectacle." Among the numerous adults swept from white clover at Kenscoff were a few nymphs, which may of course have been feeding on the grass interspersed with the clover and not on the clover itself. It is hardly possible, however, that the numerous adults could have confined their feeding to the scanty grasses present in the dominantly white clover areas, and clover may be considered as a normal host for this leafhopper at the higher elevations in the tropics where clover can maintain itself.

Of the other less numerous, less conspicuous and much smaller leafhoppers swept from white clover at Kenscoff, Dr. John S. Caldwell identified and listed in order of abundance *Unerus colonus* (Uhler), previously known on carpet grass (*Axonopus compressus*) in mountain pastures in Puerto Rico; *Deltocephalus sonorus* Ball, abundant on malojillo; *Nesosteles incisa* (Matsumara) on seed-heads of malojillo; the cosmopolitan *Exitianus obscurinervis* (Stal), and a few individuals of the light green *Empoasca sativae* Poos and *Empoasca dilitara* Davidson and DeLong. Most of these are neotropical or West Indian leafhoppers, and that they should occur on white clover in tropical Haiti would appear to indicate its introduction from France, presumably as seed, without any of its specific leafhopper pests.

The completeness with which white clover may occupy the environments to which it is particularly well adapted, to a considerable extent depends on the specific insect pests attacking it. In a pasture in northern New York, so closely cropped by cows that the recumbent habit of this clover made it the dominant plant, but a single leafhopper, *Agallia quadripunctata* (Provancher), specifically fed upon it.(2) Of this leafhopper, approximately one hundred and fifty were counted in one hundred square feet of pasture; so few that their effect on the host was practically negligible. Much more abundant was what Dr. Herbert Osborn(3) calls the meadow leafhopper. *Acucephalus nervosus* (Schrank), of which approximately five hundred nymphs and adults were present in the same hundred square feet. Bluegrass, redtop, sweet-scented grass and some timothy were present in this pasture, and may have been the normal hosts, but adults were noted resting on white clover, and possibly feeding on it. The other leafhoppers present were the timothy crown leafhopper, *Aphrodes albifrons* (L.), and in smaller numbers: *Leavicephalus affinis* (Gillete & Baker), *Polymia inimica* (Say), *Amblysellus curtisii* (Fitch) and *Balclutha impictus* (Van Duzee), most of which were so scarce as possibly having nothing to do with the white clover. Indeed, its major pests in northern New York were introduced weevils and a cutworm, of which none was noted in Haiti, and from which it may be entirely free in the tropical environment.

The only Fulgorid certainly identified from the white clover pasture in northern New York was *Bruchomorpha occulata* Newman, which was so scarce that no observation on host relationships was possible. In Haiti, Dr. Caldwell identified the most common Delphacid as *Delphacodes propinqua* (Fieber); others were *Delphacodes teapae* (Fowler), *Sogata furcifera* (Horvath) and *Euidella weedi* Van Duzee. In numbers they exceed all of the smaller leafhoppers, and in bulk were more than the approximate equivalent. The first three are more or less common neotropical inhabitants of lowland pastures and meadows, and are sometimes to be found on young sugar-cane. Their occurrence on white clover in the mountains of Haiti is only one more instance of how readily endemic insects become adjusted to flourishing, newly-introduced plant hosts, which have escaped from the pests attacking them in their country of origin.

1. Wolcott, G. N., The Minor Sugar-Cane Insects of Porto Rico. Jour. Dept. Agr. P. R., **5** (2): 5–47, fig. 19. San Juan, April 1921.

2. Wolcott, G. N., An Animal Census of Two Pastures and a Meadow in Northern New York, Ecological Monographs, **7** (1): 1–90, ref. 42. Brooklyn, January 1937.

3. Osborn, Herbert, Meadow and Pasture Insects, pp. 288, fig. 103. The Educator's Press, Columbus, 1939.

BENZENE HEXACHLORIDE AS A TERMITE REPELLENT

By GEORGE N. WOLCOTT [1]

As an experimental animal for indicating the duration or permanence of the residual effect of an insecticide or other chemical, the West Indian dry-wood termite, *Cryptotermes brevis* (Walker), is of value because its reactions are not affected by the age of the wood sample after this has become thoroly seasoned. In the evaluation of benzene hexachloride (hexachlorocyclohexane), the reactions of this termite show why this chemical may be of outstanding value in the protection of seasoning wood or bamboo against insects which normally attack it only at this time, but against the attack of which no chemical is needed a few months later when the material is completely seasoned.

Thru the courtesy of Dr. Harry F. Dietz of E. I. Dupont de Nemours & Co., a one gram sample of the pure gamma isomer of hexachlorocyclohexane was made available for test early in 1947, together with the technical 12% gamma, for comparison with a mixture of alpha and beta isomers purchased from Eastman. By comparison with a large number of other chemicals which have been tested in the laboratory, using standard technique (1), very few are as repellent to termite attack for so long a period (147 days) at a dilution of 0.05% as is the gamma isomer of benzene hexachloride. Yet, despite the continued residual resistence of the gamma isomer at greater concentrations, the sample impregnated with 2% gamma from solution in benzol, submerged ten minutes, was eventually eaten within less than a year. Because of the minute amount of the pure chemical available, no wood sample impregnated with a greater concentration than 2% of the gamma could be prepared for test. In actual commercial practice, however, if 2% of any chemical impregnated in a most susceptible wood fails to be repellent to termites over a long period of time, that chemical must be summarily dismissed as unsuitable for use as a termite repellent, valuable as it may be over shorter periods in protecting the wood against the attack of other kinds of insects. In-

[1] Head, Department of Entomology of the Agricultural Experiment Station, University of Puerto Rico

deed, as the pure gamma isomer is not commercially available now, and presumably will not become available in the near future, the use of the less repellent technical grades of benzene hexachloride for wood preservation against termite attack is not to be recommended.

The records of some other chemicals used, or suggested for use as termite repellents, are given in the accompanying table, for comparison with benzene hexachloride.

TABLE NO. I

DAYS AFTER SUBMERSION TEN MINUTES IN SOLUTION BEFORE ATTACK BY THE WEST INDIAN DRY-WOOD TERMITE, *CRYPTOTERMES BREVIS* (Walker)

Dilutions of	0.05%	0.1%	0.2%	0.5%	1%	2%
Benzene Hexachloride:						
alpha & beta (Eastman)	4	5	7	10	15	44
12% gamma (DuPont)	23	37	44	91	260	264
100% gamma (DuPont)	147	189	249	259	303	334
D D T	26	27	29	35	37	uneaten over 4 years
Pentachlorphenol		10	13	273	337	uneaten over 4 years
Copper Pentachlorphenate	108	111	uneaten 40 months			
Tetrachlorxanthone	7	114	uneaten over a year			
Zinc Lake of Alizarin	5	uneaten over a year				

1. Wolcott, G. N., "Termite Repellents: a Summary of Laboratory Tests" Bulletin No. 73, Agr. Expt. Station, Univ. P. R., pp. 18, ref. 11. Río Piedras, October 1, 1947.

THE SYNONOMY OF *EMPOASCA FABALIS* DE LONG (CICADELLIDAE: HOMOPTERA)

JOHN S. CALDWELL
Circleville, Ohio

Empoasca fabalis DeLong[1] was described from material collected April 15 and June 18, 1929 by Dr. R. C. Smith at Port-au-Prince, Haiti, and reported by him as "extremely abundant upon beans and sweet potatoes". Three years later *Empoasca batatae*[2] Poos was described from material reared on sweet potato in a green house at Arlington Farms, Virginia, and from material collected on the same host at Fort Myers, Florida. Subsequently the two names have remained in literature and have caused some confusion because only one species is involved. *Fabalis* DeL. has been reported as the most abundant form on beans in Puerto Rico. Recently Dr. Luis F. Martorell and myself spent over five months collecting *Empoasca* in Puerto Rico. The common form we found on beans proved to be *fabae* (Harris). The form taken on sweet potato and morning glory vines I identified as *batatae* Poos because the drawing of that form is more typical than the drawing for *fabalis* DeL. This raised the question and the suspicion that *batatae* was the same species as *fabalis*. After I took *batatae* (really *fabalis*) from morning glory at Port-au-Prince, Haiti, the type locality of *fabalis*, I was convinced that the two were the same species. As the request of Dr. G. N. Wolcott, Monsieur Léonce Bonnefil fils, Head of the Section of Entomology, Departement de l'Agriculture, Republique d'Haiti, forwarded two topotype collections from Damien, near Port-au-Prince, Haiti. The specimens from sweet potato were practically pure *fabalis* DeL. and the forms from bush beans were *fabae* (Harris). Later I have learned that Dr. Ralph H. Davidson considers *batatae* Poos to be a synonym of *fabalis* DeL. but has never published the fact, so I take this opportunity to do so now.

1. Can. Ent. 62:92. 1930.
2. Proc. Ent. Soc. Wash. 35:176–177. 1933.

A STUDY OF THE EFFECT OF SIX QUALITATIVE CHARACTER PAIRS ON YIELD AND COMPONENT CHARACTERS IN THE F_2 OF A CROSS BETWEEN TWO VARIETIES OF BARLEY[1]

By F. MARIOTA-TRÍAS[2]

INTRODUCTION

This article reports the relationship in barley between six qualitative character pairs and the quantitative characters: weight per seed, number of heads per plant, number of seeds per head, and yield per plant in the F_2 segregating population of a cross between the Nigrillaxum (*Hordeum distichon* L.) and Brachytic chlorina (*H. vulgare* L.) varieties of barley. The six pairs of genes studied differentiated 2-row *vs.* 6-row type of spike (V,v); black *vs.* white color of glumes (B,b); covered *vs.* naked caryopsis (N,n); hooded *vs.* awned type of lemma (K,k); normal *vs.* brachytic habit of growth (Br,br); and green *vs.* chlorina type of seedling (Fc,fc). The six contrasting characters of the parents were as follows:

$$\begin{array}{l}\text{Nigrillaxum} \\ \text{brachytic-chlorina}\end{array} = \frac{\text{V B n K Br Fc}}{\text{v b N k br fc}}$$

The data used for the study were taken by the late Dr. F. R. Immer, on material grown at University Farm, St. Paul. The quantitative data were taken on the individual F_2 plants and their genotypes were determined by F_3 progeny trials. The study consisted primarily of comparisons of the different genotypes for a single factor pair with respect to the quantitative characters. Similar comparisons were made of the interactions between factor pairs considered two at a time.

The mode of inheritance and linkage relations of the six factor pairs used are summarized by Robertson, Wiebe and Immer (13). Each of the characters is simply inherited and all are reported to be independent except br-fc which is linked

[1] Contribution from the Division of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minn. Scientific Journal Series, Minnesota Agricultural Experiment Station. Part of a thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science. Dec. 1946.

[2] Assistant Plant Breeder, Agricultural Experiment Station of the University of Puerto Rico.

with about 9.8 per cent recombination. Two of the factor pairs namely 2-row vs. 6-row (11) and hooded vs. awned (10), have been reported to show a relation to yield. The recessive characters brachytic and chlorina are usually less vigorous than the normal.

Powers (11) reported the effect on quantitative characters of 3 of the 6 character pairs in a cross between *Hordeum deficiens* (Steud) and *H. vulgare* (L.). He found that the BB and bb genotypes did not differ significantly but that Bb was in general different from the two homozygotes. The vv plants yielded more than the VV and Vv; and the Vv more than the VV plants. Normal plants of the Br Br and Br br genotypes were higher in yield than the homozygous br br.

The reader may consult Smith (14) for a comprehensive review of the literature on quantitative inheritance and Hayes and Immer (6) for a review of quantitative inheritance in barley.

Methods

The F_2 plants were grown in 1938, single seeds being planted five inches apart in rows one foot apart in a plot 18 ft. by 39 ft. in size. Three rows of each parent were planted as checks.

Notes were taken on the phenotype of each plant for the six character pairs, each plant was harvested individually and the number of heads per plant was recorded. The plants were threshed, the number of seeds counted and the yield in grams of seed determined, from which the average number of seeds per head and the average weight per seed were calculated.

Since the absence of hulls leads to a reduced weight per seed, corrections were made on the yield data for the hulles plants based on a regression between seed weight and hull weight determined from 25 seeds of each of 48 plants representing a wide range of phenotypic combinations.

The complete genotypes of the F_2 plants were determined by classifying the F_3 progeny. Of the F_2 plants, 6 of the Br Fc, 12 of the br Fc, 4 of the Br fc, and 94 of the br fc produced insufficient seed for the F_3 test (less than 0.5 grams) and were discarded leaving a total of 1044 plants.

The complete quantitative data and their squares, and the genotypes for each of the 1044 plants were punched on cards. Ordinarily the cards would then have been sorted by genotypes

to obtain the data for each gene pair singly and also in combinations of two or more gene pairs at a time. In this case, since a large proportion of the F_2 plants with insufficient seed were *br, fc* or *br fc*, making the yield of these genotypes higher than they should be, it was deemed necessary to remove the effects of the Br, br and Fc, fc segregation by including their different observed genotypes with each of the genotypes for the gene pair or pairs being studied. Consequently the cards were sorted for all genotypic combinations between the gene pair or pairs being studied and Br, br, Fc, fc.

In order to study the effects of the qualitative genes on each quantitative character, the mean value for each genotype was calculated and used in the comparisons. For example, the difference Vv-vv was interpreted as the effect of substituting the first dose of the dominant gene; VV-Vv the effect of substituting the second; and VV-vv the total effect of both. In studying the interactions between two non-allelic genes, for example, V,v with B,b; the above three comparisons were made in each of the BB, Bb, and bb genotypes. Finally, the effect in one of these latter genotypes was compared with that in each of the other two. For example the Vv-vv effect in the BB genotype was compared with that in the Bb and bb genotypes.

The statistical methods for calculating the means and standard errors and for determining the significance of differences will now be described.

Statistical Methods

The method of removing the effects of the Br, br and Fc, fc segregation will be illustrated by the calculation for the yield of a genotype used in the study of the interaction between V,v and B,b. To obtain the corrected sum of squares of the yields for the VVBB genotype, that is, freed from the effects of segregation for Br, br and Fc, fc, an analysis of variance for individual plant yield for each of the different genotypic combinations between (Br *vs.* br) (Fc *vs.* fc) within the VVBB genotype was calculated separately and the totals combined. The formula used is as follows:

"the sum of squares" $= S(y)^2 - S(y)\bar{y}$, where: $S(y) =$ sum of the yields of all plants, $\bar{y} =$ mean yield of the plants of the given genotype and $y =$ yield of individual plants.

The results of the calculations for the VVBB genotype are given in Table 1. Since there are 69 plants in the VVBB segregation composed of 7 classes for Br, br *vs.* Fc, fc, the degrees of freedom will be 69-7 = 62. The total sum of squares, 350.75, divided by the degrees of freedom, 62, is the variance or mean square. The variance of the mean was determined from the formula: $Vm = \frac{\text{Total sum of squares.}}{\text{D.F x N}}$ This method of calculating the variance combines the differences between the genotypic combinations between Br, br and Fc, fc into a single value, this being used to calculate a generalized estimate of the error for that particular genotype of V with B.

TABLE No. I

ILLUSTRATION OF THE METHOD OF CALCULATING THE CORRECTED SUM OF SQUARES OF THE YIELDS OBTAINED WITH A GIVEN GENOTYPE (VVBB) FOR ALL COMBINATIONS OF THE QUALITATIVE CHARACTERS Br br AND Fc fc[1]

Genotype	N	$S(y^2)$	$S(y)(\overline{y})$	$S(y^2)-S(y)(\overline{y})$
VVBBBrBrFcFc	17	745.83	613.20	132.63
VVBBBrBrFcfc	5	243.24	233.93	9.31
VVBBBrBrfcfc				
VVBBBrbrFcFc	5	246.36	228.49	17.87
VVBBBrbrFcfc	31	1,265.67	1,079.11	186.56
VVBBBrbrfcfc	2	3.94	3.92	.02
VVBBbrbrFcFc				
VVBBbrbrFcfc	4	18.78	15.21	3.57
VVBBbrbrfcfc	5	5.99	5.20	.79
	69			350.75*

* Corrected sum of squares for VVBB.

[1] The method of removing the effects of Br br vs. Fc fc was suggested by W. G. Cochran in a letter to Dr. F. R. Immer.

TABLE No. II

ILLUSTRATION OF THE METHOD OF CALCULATING THE MEAN SQUARES, AND VARIANCE OF THE MEAN USING "SUM OF SQUARES" CORRECTED BY REMOVING THE Br br VS. Fc fc EFFECTS

Genotype	N	Classes	D. F.	Mean Yield	Corrected Sum of Squares	Mean Square	Variance of mean
VVBB	69	7	62	5.343	350.75	5.6572	.081989
VVBb	122	7	115	5.163	969.01	8.3392	.068354
VVbb	79	7	72	5.589	791.72	10.9961	.139191
VvBB	144	9	135	6.356	1,217.66	9.0197	.062637
VvBb	267	7	260	5.745	2,961.78	11.3915	.042665
Vvbb	114	8	106	5.818	960.81	9.0642	.079511
vvBB	62	7	55	6.082	614.19	11.1671	.180114
vvBb	116	7	109	6.217	1,278.07	11.7254	.101081
vvbb	71	8	63	5.139	740.58	11.7552	.165567
	1,044	67	977		9,874.57		

Table 2 summarizes the calculations for the nine genotypes for the factor pairs V,v and B,b. To determine if a single generalized error could be used for the 9 genotypes insolving V,v and B,b, a homogeneity test (1) was applied to the 9 separate generalized errors. The writer found heterogeneity as Powers (11) did in his study. Since the variance were not homogeneous, and a generalized estimate of error could not be used, "t" values to determine the significance of mean differences between genotypes were calculated by the usual formula for $s_{\bar{d}}$, the standard error of a difference between two means: $s_{\bar{d}} = \sqrt{V_1 + V_2}$, where V_1 and V_2 are the respective variances of the means under comparison.

As an example, the effect of VV will be compared with that of Vv in the BB genotype, taken from Table 2. For VVBB, $V_1 = 0.081989$ and VvBB, $V_2 = 0.062637$.

Then $s_{\bar{d}} = \sqrt{0.081989 + 0.062637} = .3803$

Since the ratio of the difference between means, $\bar{d}$, to its standard error, $s_{\bar{d}}$, does not follow Student's "t" distribution except in special instances (2), some other method must be used. The approximation t′ given by Cochran and Cox (2) was used. The approximation consists in calculating $t = \frac{\bar{d}}{s_{\bar{d}}}$ and finding a significance level t′ weighted in accordance with the following procedure:

$$t' = \frac{w_1 t_1 + w_2 t_2}{w_1 + w_2}$$ * where t_1 and t_2 are the significance level values for n_1 and n_2 degrees of freedom and $w_1 = V_1$ and $w_2 = V_2$, the latter being the variances of the two means. In the present example for VVBB-VvBB at the 5% level:

$$t' = \frac{(.081989 \times 1.995) + (.062637 \times 1.977)}{.081989 + .062637} = 1.987.$$

* Since $w_1 = V_1$ and $w_2 = V_2$, this formula becomes $t' = \frac{V_1 t_1 + V_2 t_2}{V_1 + V_2}$

Since t' always lies between the ordinary t values for n_1 and n_2 degrees of freedom, all cases except those on the borderline of significance can be decided without the actual calculation of t'. Since the difference in mean yields is:

VvBB — VVBB = 6.356 — 5.343 = 1.013,

$$\text{then } t = \frac{\bar{d}}{s_{\bar{d}}} = \frac{1.013}{.3803} = 2.664$$

In this example the observed "t" value of 2.664 is well above not only this t' value, but also the "t" value of 1.995 corresponding to the 5% level for 62 degrees of freedom. Therefore it would not have been necessary to calculate t' for this comparison.

For the effects of the different genotypes for each gene pair considered singly, the mean value of the corresponding quantitative character for each was calculated and the corresponding variance of the mean determined by using the corrected "sum of squares" as described above to remove the effects of the Br, br and Fc, fc segregation.

The net effects of V *vs.* v in the population classified for each of the other gene pairs considered singly were also calculated from data in which the interactions were studied. For example, for the V-v pair in the population classified for B *vs.* b the following values were calculated from the data in table 2.

Genotype	N	D. F.	Totals of yields	Corrected sum of squares	Variance of the mean
VV	270	249	16.095	2,101.48	.031258
Vv	525	501	17.919	5,140.26	.019543
vv	209	227	17.458	2,632.84	.046580

For the net effect of adding one V, we have

Vv-VV = 17.919 — 17.458 = .461 (net effect),

and $t = \frac{.461}{\sqrt{.019543 + .046580}} = 1.794$, significance being determined as before. In general these net effects should agree with the effects of each gene based on the total population. Any differences result from the fact that the net effects are based on means for genotypic combinations between two pairs of factors, the grouping being different for different cases. In determining the effects of the Br-br and Fc-fc gene pairs con-

sidered singly, mean values and their variances were calculated for each quantitative character directly in spite of the possible bias due to the elimination of a large number of the recessives. Had they been included, the differences would have been still larger.

Interactions (To determine whether a gene effect in one of the three genotypic backgrounds for a second gene pair was significantly different from its effect in either of the other two genotypic backgrounds for this gene pair).

Since an analysis of variance could not be calculated to test the significance of the interactions of one gene with a non-allele, the "t" values to determine the significance of the first order interactions were obtained by the method given by Powers (12). The formula for obtaining t as stated by him is as follows:

$$t = \frac{\bar{x}_1 + \bar{x}_4 - \bar{x}_2 - \bar{x}_3}{\sqrt{\frac{S_1^2}{n_1 n_1'} + \frac{S_2^2}{n_2 n_2'} + \frac{S_3^2}{n_3 n_3'} + \frac{S_4^2}{n_4 n_4'}}} \quad \text{or} \quad \frac{\bar{x}_1 + \bar{x}_4 - \bar{x}_2 - \bar{x}_3}{\sqrt{V_1 + V_2 + V_3 + V_4}}$$

In the above formula $\bar{x}_1$, $\bar{x}_2$, $\bar{x}_3$ and $\bar{x}_4$ are the mean of the different genotypes: S_1^2, S_2^2, S_3^2 and S_4^2 are the sum of squares for the estimation of the separate errors; n_1, n_2, n_3 and n_4 are the corresponding degrees of freedom available for an estimation of the separate errors; and n'_1, n'_2, n'_3, and n'_4 are the corresponding total numbers of individuals for the genotypes whose means are involved in the calculation of the interaction.

As an illustration of this calculation of interaction, the following data for yield from Table 2 may be used: (Vv BB — vvBB) — (Vv Bb — vv Bb) = (6.356—6.082) — (5.745—6.217). In other words the Vv-vv effect is being compared in the BB and Bb backgrounds.

$$t = \frac{6.356 + 6.217 - 6.082 - 5.745}{\sqrt{\frac{1217.66}{(135\text{x}144)} + \frac{614.19}{(55\text{x}62)} + \frac{2961.78}{(260\text{x}267)} + \frac{1278.07}{(109\text{x}116)}}}$$

$$= \frac{6.356 + 6.217 - 6.082 - 5.745}{\sqrt{0.062637 + 0.180114 + 0.042665 + 0.101081}} = \frac{.746}{.6217} = 1.200$$

For these "t" tests of significance, all values found to exceed 1.960 were considered significant at the 5% point, since the total number of degrees of freedom in each test was always very large.

The mean, variance, variance of the mean and the standard errors of the yields and its components were calculated for the six character pairs singly and in all combinations of two each. Differences giving probabilities as low or lower than 0.05 that the deviations as great as those noted might arise because of errors of random sampling were considered to be statistically significant; although some caution must be exercised since with such a large number of comparisons, certain of them are undoubtedly chance variations.

Experimental Results

Each factor pair except Fc-fc in F_2 and all of them in F_3 gave good agreement with a monogenic ratio as expected from previous studies (13). For the Fc *vs.* fc pair there was a deficiency of chlorina plants in F_2 due possibly to low germination or poor survival.

The X^2 values for the linkage components indicated no association except between Br,br and Fc,fc factor pairs and between N,n and K,k. All other combinations of two factor pairs showed a good fit to dihybrid segregations. The F_2 data on which these are based are in table 3.

Table No. III

F_2 SEGREGATIONS FOR CHARACTERS SHOWING LINKAGE, F_2 DATA

Segregation	AB	Ab	aB	ab	Total	Linkage Component		Per Cent Recombination Product Method	Weighted p value F_2 and F_3 Data
						X^2	P		
N-K	575	200	210	59	1,044	1.63	.30—.20	47.0±2.40	46.1±1.71
Br-Fc	800	21	32	131	1,044	376.10	.0001	6.3± .78	7.6± .58

The loose but significant linkage btween N and K suggests a chromosome abnormality, possibly a translocation, but was not found in time for a cytological study. The interpretations of the results may be affected since two closely linked qualitative factors might be expected to show similar associations with linked quantitative factors.

Main Effects—Differences Between Genotypes for Each Gene Pair Considered Singly

The average values for yield and its components for the homozygous and the heterozygous genotypes are in table 4 and the comparisons for each gene pair are in table 5. Any observed difference may be interpreted either as due to the effect of the gene itself or to the effect of one or more linked quantitative factors. If the latter, the observed degree of effect will depend on the closeness of the linkage, i.e. the closer the linkage the more nearly will the observed difference represent the actual effect of the quantitative gene itself. For ease of discussion the difference will be described as due to the effect of the qualitative factor itself throughout the presentation of the results, although in reality it may be due to an association with a quantitative factor.

The comparisons between genotypes for a given gene pair, for example Vv-vv, VV-Vv, and VV-vv, are interpreted as being the effect of substituting the first dominant, the second dominant, and both respectively for either or both recessive alleles. The effect of the second dominant is the effect of adding another one to a genotype which already has one. The VV-vv difference is the algebraic sum of the other two.

Table No. IV

A SUMMARY OF QUANTITATIVE CHARACTER MEANS FOR THE DIFFERENT GENOTYPES CONSIDERED SINGLY

Genotype	N	Mean yield in grams of seeds per plant	Mean number of heads per plant	Mean number of seeds per head	Mean weight of seed in grams per seed
VV	270	5.334	9.211	154.65	.032384
Vv	525	5.929	9.265	223.46	.025778
vv	249	5.882	7.924	261.59	.021481
BB	275	6.040	9.516	225.12	.026812
Bb	505	5.713	8.824	213.51	.026514
bb	264	5.372	8.527	206.34	.025997
NN	276	5.832	8.887	222.70	.026170
Nn	490	5.674	8.894	211.25	.026344
nn	269	5.859	9.045	213.15	.026979
KK	264	5.153	9.015	197.11	.025725
Kk	521	5.739	9.142	214.13	.026678
kk	259	6.455	8.420	234.00	.026776
BrBr	360	6.639	10.006	253.81	.026681
Brbr	521	6.602	9.994	236.73	.028003
brbr	163	1.148	3.160	58.26	.019154
FeFe	333	6.585	10.048	248.92	.026952
Fefe	559	6.601	9.998	240.46	.028110
fefe	152	.885	2.671	45.39	.019354

TABLE NO. V

DIFFERENCES IN QUANTITATIVE CHARACTER MEANS BETWEEN GENOTYPES FOR INDIVIDUAL CHARACTER PAIRS CONSIDERED SINGLY

Genotype	Difference between mean yields	Difference between mean number of heads	Difference between mean number of seeds	Difference between mean weight of seed
Vv-vv	.047	1.341**	—38.13**	.004297**
VV-Vv	—.595*	—.054	—68.81**	.006605**
VV-vv	—.548*	1.287**	—106.95**	.010903**
Bb-bb	.141	.297	7.17	.000517
BB-Bb	.327	.692*	11.61	.000298
BB-bb	.468	.989**	18.78	.000815
Nn-nn	—.185	—.151	—1.90	—.000635
NN-Nn	.158	—.007	11.45	—.000174
NN-nn	—.027	—.158	9.55	—.000809
Kk-kk	—.726**	.722*	—19.87*	—.000098
KK-Kk	—.576**	—.127	—17.02*	—.000953*
KK-kk	—1.302**	.595	—36.89**	—.001051*
BrBr-brbr	5.454**	6.834**	178.47**	.009450**
BrBr-Brbr	.037	.012	17.08	—.001920**
BrBr-brbr	5.491**	6.846**	195.55**	.007530**
FcFc-fcfc	5.716**	7.297**	195.07**	.008760**
FcFc-Fcfc	—.016	.080	8.46	—.001160*
FcFc-fcfc	5.700**	7.377**	203.53**	.007600**

* Significant at the 5% probability level.
** Significant at the 1% probability level.

Effect of 2-row versus 6 row (*V vs. v*). There was no effect on yield due to the substitution of the first dominant as measured by the Vv-vv comparison. The substitution of the second V as measured by the VV-Vv comparison significantly decreased yield. The total effect of substituting both V's as measured by the VV-vv comparison was a significant decrease in yield.

The substitution of the first V significantly increased the number of heads per plants, the second V had no effect, while the total effect of both was to significantly increase the number of heads.

For number of seeds per head the first V produced a decrease, the second V reduced it still further, and the total effect of both was necessarily a significant decrease.

As to average weight per seed the first V increased it significantly, the second V increased it still further and again the effect of both was, as expected, a significant increase.

The effect of the first V to increase number of heads and weight per seed was counterbalanced by a decrease in number of seeds, the net result being no effect on yield. The substitution of the second V had no further effect on number of heads,

but decreased the number of seeds and increased the weight per seed. The decrease in number of seeds per head accounts for the net decrease in yield as a result of substitution of the second V. These comparisons thus represent the change from 6- to 2-row.

Effect of black versus white color of glumes (*B vs. b*). There was no effect on yield, number of seeds per head nor weight per seed due to the substitution of the first or the second B or of both.

For number of heads per plant the substitution of the first B had no effect. However, the second B significantly increased the number of heads per plant, and the total effect of both B's was a significant increase. In spite of this, there was no significant net effect on yield.

Effect of covered versus naked caryopsis (*N vs. n*). No one of the quantitative characters was affected by substituting either the first or second N or both.

Effect of hooded versus awned lemma (*K vs. k*). The substitution of the first K significantly decreased the yield, the second K decreased it still further, while the effect of both K's was necessarily a significant decrease.

There was an increase in number of heads per plant due to the substitution of the first K.

As to number of seeds per head the first K decreased it significantly, the second K decreased it still further, while the total effect of both K's was a significant decrease.

For weight per seed the first K had no effect, while the substitution of the second K and of both K's significantly decreased it.

The significant decrease in yield due to the substitution of the first K appears to have been brought about mainly by a decrease in seed number, since the number of heads was actually increased. The decrease in yield attributed to the second K seems to have been the result of a decrease in weight of seeds as well as in number of seeds.

Effect of normal versus brachytic habit of growth (*Br vs. br*). The substitution of the first Br produced a significant in-

crease in the yield, number of heads, number of seeds and weight per seed. The second Br had no effect, except to decrease the weight per seed. The total effect of both was a significant increase in yield, number of heads, number of seeds and weight per seed. As to weight per seed the heterozygous Br br seeds were heavier than either of the homozygotes, an indication of heterosis or of Hull's superdominance (7).

Effect of normal versus chlorina seedlings (*Fc vs. fc*). The substitution of the first Fc produced a significant increase in the yield, number of heads per plant, number of seeds, and weight per seed. The second Fc had no effect except to decrease weight per seed. The total effect of both F'cs was a significant increase in yield, number of heads, number of seeds, and weight per seed. The heterozygous (Fcfc) seeds were heavier than the homozygous ones, again an indication of heterosis.

Interactions of each gene pair with a non-allelic pair. Two questions will be considered here: first, in which genetic backgrounds is a given gene effect significant; and second, are there effects which are significantly different in the different genotypes for a given non-allelic gene pair. For brevity, only the data on the interactions of the V-v pair with non-alleles are presented, information on the first question being in table 6. Information on the second question is in table 7. It is helpful in interpreting the latter tables to refer to the corresponding tables 6 and 7 to determine the magnitude and direction of the differences being compared. For example, the significant interaction (VVBb-vvBb)—(VVbb-vvbb) = —1.484 (table 7) is a comparison between (VVBb-vvBb) = —1.054 and the (VVbb-vvbb) = .430 given in table 6. Interactions of the other gene pairs will be discussed without presenting the data.

TABLE No. VI

QUANTITATIVE CHARACTER DIFFERENCES BETWEEN GENOTYPES FOR THE V vs. v CHARACTER PAIR WITHIN DIFFERENT GENOTYPES FOR A NON-ALLELIC PAIR

	Yield				Number of heads		
	B vs. b	N vs. n	K vs. k		B vs. b	N vs. n	K vs. k
				Vv-vv			
AA	.274	—.073	—.098		2.032*	.967	1.590**
Aa	.472	.136	.361		.541	1.550**	1.335**
aa	.659	.044	—.291		1.919*	1.395**	1.085
				VV-Vv			
AA	—1.013*	1.570**	—.220		—.488	—.482	—.122
Aa	—.582	—.555	—.430		—.137	—.205	—.119
aa	—.229	.149	—1.308*		.553	.550	.252
				VV-vv			
AA	—.739	—1.643**	—.318		1.544*	.485	1.378*
Aa	—1.054*	—.419	—.069		.404	1.345**	1.246**
aa	—.436	.193	—1.509*		2.472*	1.945**	1.337

	Number of seeds				Weight per seed		
	B vs. b	N vs. n	K vs. k		B vs. b	N vs. n	K vs. k
				Vv-vv			
AA	—22.65	—49.90*	—41.69*		.033331**	.035938**	.034788**
Aa	—65.91**	—31.06*	—32.74**		.034573**	.033443**	.034395**
aa	—6.44	—37.09	—40.83		.034364**	.033924**	.033570**
				VV-Vv			
AA	—83.78**	—88.50**	—53.62**		.037229**	.033814**	.035941**
Aa	—64.73**	—71.80**	—61.98**		.036254**	.037516**	.037060**
aa	—62.68**	—46.50**	—98.50**		.036845**	.037458**	.036635**
				VV-vv			
AA	—106.43**	—138.50**	—95.31**		.010560**	.009752**	.010729**
Aa	—130.64**	—102.36**	—94.72**		.010827**	.010959**	.011455**
aa	—69.12**	—83.68**	—139.35**		.011209**	.011382**	.010295**

TABLE No. VII

SIGNIFICANT INTERACTIONS OBTAINED BETWEEN A GIVEN EFFECT OF V vs. v AND DIFFERENT GENOTYPES FOR A NON-ALLELIC PAIR

Interactions	Difference
YIELD PER PLANT:	
(VVNN-VvNN)—(VVnn-Vvnn)	—1.719**
(VVNN-VvNN)—(VVNn-VvNn)	—1.015*
(VVNN-vvNN)—(VVnn-vvnn)	—1.836*
(VVBb-vvBb)—(VVbb-vvbb)	—1.484*
AVERAGE NUMBER OF HEADS PER PLANT:	
(VvBB-vvBB)—(VvBb-vvBb)	1.491*
(VvBb-vvBb)—(Vvbb-vvbb)	—1.378*
(VVBb-vvBb)—(VVbb-vvbb)	—2.068*
AVERAGE NUMBER OF SEEDS PER HEAD:	
(VvBb-vvBb)—(Vvbb-vvbb)	—59.47*
(VVNN-VvNN)—(VVnn-Vvnn)	—41.91*
(VVKK-VvKK)—(VVkk-Vvkk)	44.88*
(VVBb-vvBb)—(VVbb-vvbb)	—61.52**
AVERAGE WEIGHT PER SEED:	
(VvNN-vvNN)—(VvNn-vvNn)	.002405**
(VVNN-VvNN)—(VVNn-VvNn)	—.003702**
(VVNN-VvNN)—(VVnn-Vvnn)	—.003644**

Interactions of V-v. For average yield per plant, table 6 shows that the substitution of the first V had no significant effect in any of the genotypes of B-b, N-n, and K-k. The second V significantly decreased the yield only in the BB, NN and kk genotypes. The effect of both V's was significant only in the Bb, NN, and kk genotypes. Table 7 shows that the effect on yield of the second V, as measured by VV-Vv, was significantly different in the NN as compared with either the Nn or nn genotypes. The effect of both V's was significantly different in the NN *vs.* nn; and also in the Bb *vs.* bb genotypes. All other effects on yield were not significantly different in the three genotypes for any given non-allelic pair.

For average number of heads per plant, (table 6), the substitution of the second V was in no case significant in any of the non-allelic backgrounds. Substitution of the first V resulted in a significant increase in all cases except in the Bb, NN and kk genotypes. The effect of both V's was also significant in all cases except in these same genotypes. The effect of the V *vs.* v for number of heads is only significantly greater, (table 7), in the BB and bb genotypes than in Bb. The effect of both V's in bb was greater than in Bb.

In the study of average number of seeds per head, the first V produced a significant decrease, (table 6), in the Bb, NN, Nn, KK and Kk genotypes. The second V produced much greater reductions which were highly significant in all genotypes. The addition of both V's was also significant in all genotypes. The decrease due to the first V was greater, (table 7), in the Bb than in the bb genotype. The further decrease due to the second V was greater, in the NN than in the nn genotype and in the kk than in the KK genotype. The decrease due to both V's was greater in the Bb than in the bb genotype.

For average weight per seed, the first V brought about a highly significant incresae in all cases (table 6). The second V still further increased the seed weight by a highly significant amount in all cases. The increase due to the first V was greater, (table 7), in the NN than in the Nn genotypes. The increase due to the second V were greater in the NN than either the Nn or the nn genotypes.

The chief component to account for the decreased yield in certain genotypes was the decrease in average number of seeds per head, since seed weight was increased in all genotypes.

Interactions of K vs. k. For average yield per plant, the effect of the first K was to significantly decrease yield in the Vv, vv, Bb, Nn and nn genotypes (all P* except in nn, P**). Addition of the second K decreased the yield only in the Vv, Bb and nn genotypes. The total effect of both K's was to decrease the yield in the Vv, vv, Bb, Nn and nn genotypes (P** for all but vv and Nn for which P*). The effect in yield of the first K was significantly different in the NN *vs.* nn genotypes (P**). The effect of the second K was different in the Bb and BB genotypes (P*) and in the Nn and nn genotypes (P**). The total effect of both K's was significantly different in BB and Bb (P*), NN *vs.* Nn and nn genotypes (P**).

For number of heads per plant, the effect of the first K, the second K and of both was not significant in any genotype. The total effect of both K's was different in the Nn and nn genotypes (P*).

For number of seeds per head, the effect of the first K was negative and significant (P*) in the Vv, and nn genotypes. The effect of the second K was significant in the Bb and nn genotypes. The effect of both was significant (P**) in the Vv, Bb and nn genotypes. The total effect of both K's was different in VV and Vv and in NN and nn (P*) genotypes.

For weight per seed the first K had no significant effect. The second K decreased the weight (P*) in the VV and vv genotypes. The total effect of both was a decrease in weight in the vv, Bb and nn genotypes. The effect of the first K was different in the NN and nn genotypes (P*). The effect of both K's was different in NN and nn genotypes (P*).

Interactions of B vs. b. For average yield per plant there was a significant increase in yield of BB over bb (P*) in the NN genotype, and in the vv genotype for the Bb-bb (P*) comparisons. In the BB-Bb comparisons there were significant differences in the KK (P**) genotype. There appeared to be a trend in favor of higher yield of the BB genotype, although this may be due to chance, since the net difference was not significant. The effect on yield of the first B was greater (P*) in the vv than in the VV genotype. The second B effect was greater (P*) in the KK than in either the Kk or kk genotypes.

For average number of heads per plant, significant differences in favor of BB over Bb plants were found in the Vv, Nn

P* means that the difference so qualified was significant at the 5% point.
P** means that the difference so qualified was significant at the 1% point.

and KK genotypes. In the BB-bb comparisons, there were significant differences in favor of the BB genotype in Vv, NN and Nn. The effect of the first B was greater (P*) in the vv than in either the Vv or the VV genotypes. The effect of the second B was greater in the Vv than in the vv genotype (P*).

For number of seeds per head, the effect of the first B was a significant increase (P*) in the vv genotype. The effect of adding the second B was an increase only in the Vv, and KK genotypes, (P*). The total effect of both was significant only in the NN genotype (P*). The effect of the first B was greater in the vv than in either the VV or the Vv genotype.

For average weight per seed, the increase due to the first B was significant only in the NN genotype (P*). The effect of the second B was significant in no case. Only in the vv genotype was the total effect of both B's significant (P*) while the individual effects in this vv genotype were not. The effect of the first B was greater (P*) in the NN than in the nn genotype.

N vs. n interactions. For average yield per plant, the addition of the first N produced no significant effects. The same was true for the addition of the second N. The total effect of both was significant and negative in the VV (P**) genotype, positive in the KK (P*) and negative again in the kk genotype. In other words, the total effect of both was to depress the yield in the presence of kk; and to increase it in the presence of KK. The effect on yield of the first N was to increase the yield in the KK genotype and decrease it in the Kk and kk genotypes. The effect of the second N was to increase the yield VV and to decrease it in the Vv. The effect of both N's was different in the VV than in the Vv and vv (P*) and in the kk from that in either the KK (P**) or the Kk (P*) genotype.

For number of heads per plant, in no case was there any specific interaction with a non-allelic genotype. The effect of the first N was different in the kk genotype where there was a decrease in number of heads while in the KK there was an increase in number of heads.

For number of seeds per head, in the VV genotype the total effect of both N's was to significantly decrease the number of seeds per head (P*). The effect of the first N was different in the KK from that in the kk genotype (P*). The effect of

both N's was significantly different in the VV from that in the Vv genotype (P*) and in the KK from that in the kk genotype (P*).

For average weight per seed, addition of the first N significantly (P*) decreased weight per seed in the bb genotype. Addition of the second N affected seed weight in the VV (P*) and Vv (P**) genotypes. In the former the weight was decreased, in the latter it was increased. The total effect of both N's was significant and negative in the VV (P**), bb (P*), and kk (P**) genotypes. The effect of the second N was different in the Vv from that in either the VV or vv genotypes (P**). The effect of both N's was different in the VV from that in the Vv genotype (P**); in the bb from the Bb genotype (P*) and in the kk from that in either the KK or Kk genotypes (P*).

Discussion of Results

Before attempting to interpret the differences found to be statistically significant, it is necessary to point out that in such a large number of comparisons as was made in this study, a few of them are actually only chance differences. Which ones they are, cannot be recognized. To be safe, those which do not appear to be consistent are regarded with suspicion. In a study of sampling using Student's distribution, Treloar and Wilder (15) studied this problem to which the reader is referred if interested.

Certain of the qualitative characters were consistently associated with differences in the quantitative ones, and will be discussed here. Many of the associations can be interpreted as results expected from the known morphological or physiological differences brought about by the qualitative characters.

The higher yield of awned lemma (kk) over hooded (KK) is probably due to the favorable physiological effect of the presence of the awn which has been reported by many workers (review in Hayes and Garber (5)). The higher yield of the 6-rowed (vv) over the 2-rowed (VV) plants may be accounted for by a greater number of seeds per head since the laterals of the 2-row plants (VV) are infertile.

A higher number of seeds per head, one of the major components of yield, was found associated not only with the vulgare type of spike (vv) but also with awned lemma (kk). The

latter does not naturally follow except possibly from a physiological effect of the awn. The higher weight per seed associated with 2-row type of spike (VV) and awned lemma (kk) may have similar explanations. The seed of the 2-row types is expected to weight more since the central row seeds usually are larger than those in the laterals (8). Also the reduced seed number per head, in 2-row as compared with 6-row may result in increased seed size. The higher weight per seed of awned lemma (kk) over hooded (KK) may de due to the favorable physiological effect of the awn.

Higher number of heads per plant was associated with 2-row type of spike (VV) and with black color of glumes (BB). The latter may be due to an association of the BB character pair with quantitative factors which in their total effect produced more heads per plant.

The results obtained in this study of the cross between *Hordeum distichon* and *H. vulgare* are similar to those obtained by Powers (11) for the V,v and Br, br factors pairs. Powers found that Bb plants yielded more than either of the two homozygotes. In the present study there was no such evidence of heterosis in the B *vs.* b pair. Perhaps this latter result would lead to the conclusion that the B *vs.* b factors themselves were not the cause of the greater vigor observed by Powers.

In the present study manifestations of heterosis were found for weight per seed in the heterozygotes, Br br and Fc fc, when compared with their respective homozygous types. Similarly these results may not have been caused by the factors themselves since Powers did not obtain this. Since there is no manifestation of heterosis for yield or the other components, this one effect does not appear very conclusive. The brachytic (br br) and chlorina (fc fc) character mutations may be classified as physiological defectives according to East's (3) terminology. The brachytic and chlorina genes produce weak plants, but are not lethal. Gustafsson (4) presents a hypothesis according to which mutations in plants, many of which are lethal in homozygous condition, increase vigor when they occur in a single dose. Isogenic stocks would furnish better material for the study of the effects of the qualitative genes themselves, although even then closely-linked quantitative factors may still be present to affect the results.

In the study of quantitative inheritance there are possibilities of errors due to metrical bias. This bias is due to the inherent relation between the scale of measurements and the phenotypic expression. This may or may not have genetical significance and can be removed by an appropriate transformation of scales (9), but by so doing information on certain of the interactions is sacrificed.

Since in the present study the aim was to study the effects of the different genes and their interactions no transformation of scales to account for metrical bias was made.

SUMMARY

1. Certain statistical methods that have been used for studies of quantitative inheritance are presented together with illustrations of their application.

2. The effect of the substitution of the first dominant, second dominant and the effect of both were calculated as the following differences between genotypes: Aa-aa, AA-Aa and AA-aa respectively, the latter being the algebraic sum of the other two.

3. Significant associations between certain qualitative and quantitative characters were found. Of the significant effects, there were several in which the first dominant produced the full effect, several in which the second produced the full effect, while in a few each produced an effect.

4. The vv (6-row) genotype was higher in yield than VV (2-row) while kk (awned) was higher in yield than either Kk or KK (hooded). Higher number of seeds per head and lower weight per seed were found in vv than in either Vv or VV. The kk genotype had a higher weight per seed than KK and a higher number of seeds per head than either Kk or KK.

5. For the most part, the above associations are explainable as expected from the known morphological or physiological effects caused by the factor pairs.

6. The *Br* and *Fc* factors were associated with higher yields and were higher in all components than the homozygous recessives.

7. The N *vs.* n gene pair showed no differential effects, indicating that a high-yielding naked barley is a definite possibility.

8. When the effects of a given gene pair are considered in the different non-allelic genotypes it was found that the V *vs.* v and K *vs.* k as a general rule were very consistent in their effects in all genotypes.

9. For the other gene pairs, certain genotypes showed specific interactions with certain non-allelic genotypes. For example, the addition of the second N decreased the weight per seed in the VV while it increased it in the Vv genotype. Also the effect of N on yield was an increase in KK plants and a decrease in kk plants.

10. The nature of these interactions was such as to permit no general conclusions. While several of them may have been due to chance, it seems clear from the number obtained that such specific interactions are a part of the general picture as to the action of quantitative factors.

11. An unexpected linkage was found between K and N of 46.1 $\pm$ 1.71. A cytological study was not made to determine the cause of this unexpected result although a translocation is suspected.

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to Dr. C. R. Burnham for his advice and guidance regarding the conduct of the study and preparation of the manuscript, to Dr. H. K. Hayes for the critical reading of the manuscript, and for helpful suggestions in the interpretation of the results, and to Dr. F. R. Immer (deceased) for suggesting the problem, supplying the data and for his advice and guidance during the early phase of the study. He is also indebted to Dr. H. H. Kramer, formerly Instructor, University of Minnesota, now Associate in Agronomy, Purdue University, for his helpful suggestions dealing with the statistical analysis of the data and to Dr. J. W. Lambert for his help in the taxonomic classification of the barley varieties studied.

LITERATURE CITED

1. BARTLETT, M. S. Some examples of statistical methods of research in agricultural and applied biology. Suppl. Journ. Roy. Stat. Soc. 4:137–183. 1937.
2. COCHRAN, W. G. and COX, G. M. Experimental Designs. 150 p. 1944.
3. EAST, E. M. Genetics reactions in Nicotiana III. Dominance. Genetics 20:443–451. 1935.

4. GUSTAFSSON, A. The effect of heterozygosity on viability and vigor. Hereditas 32:263–268. 1946.

5. HAYES, H. K. and GARBER, R. J. Breeding Crop Plants. 438 p. 1927. McGraw Hill Book Co.

6. ..... and IMMER, F. R. Methods of Plant Breeding. 432 p. 1942. McGraw Hill Book Co.

7. HULL, F. R. Recurrent selection and specific combining ability in corn. Jour. Am. Soc. Agron. 37:134–145. 1945.

8. LIANG, T. J. A study of the characters of six-rowed F_4 segregates from crosses between two-rowed and six-rowed barley. M. S. Thesis. Univ. of Minn. 1946.

9. MATHER, K. Polygenic inheritance and natural selection. Biol. Rev. 18:32–64. 1943.

10. PERLITIUS, L. Der Einflus der Begrannung auf die Wasserverdunstüng der Ahren and die Kornqualitat. Inaug. Diss. Breslau. 1903.

11. POWERS, L. R. The nature of the interaction of genes affecting four quantitative characters in a cross between *Hordeum deficiens* and *H. vulgare*. Genetics 21:398–420. 1936.

12. ..... Studies on the nature of the interactions of the genes differentiating quantitative characters in a cross between *Lycopersicum esculentum* and L. *pimpinellifolium*. Jour. Genetics 39:139–170. 1939.

13. ROBERTSON, D. W., WIEBE, G. A. and IMMER, F. R. A summary of linkage studies in barley. Jour. Am. Soc. Agron. 33:47–64. 1941.

14. SMITH, H. H. Recent studies on inheritance of quantitative characters in plants. The Bot. Rev. 10:349–382. 1944.

15. TRELOAR, A. E. and WILDER, M. A. The adequacy of Student's criterion of deviations in small sample means. Ann. of Math. Statistics 5:324–341. 1935.

STUDIES ON VIRUS DISEASES OF PAPAYA (CARICA PAPAYA) IN PUERTO RICO [1]

I. Transmission of Papaya Mosaic

By JOSÉ ADSUAR [2]

INTRODUCTION

A survey of the available literature (1-14) demonstrates the presence of a serious mosaic disease of papayas (*Carica papaya*) widely spread throughout different regions of the world. In spite of this fact, surprisingly little is known as to the nature of the virus or viruses involved, means of transmission, dissemination, etc. As far as we have been able to determine, only four papers (1, 2, 6, 8) deal at any length with the problem and in only one of them (8) is there a serious claim as to the successful transmission of the disease.

In Puerto Rico, a disease of papaya known under the name of *bunchy top* was first reported in 1931 by Cook (3, 4) but it was not until 1937 that its epidemic proportions were noticed at the Isabela Substation, during the course of extensive trials conducted with the purpose of studying the possibilities of large scale commercial plantings. So severe was the attack by the following year, that the Station authorities declared the disease to be the limiting factor in the successful commercial development of this promising crop in the Island.

Almost simultaneously with the outbreak at Isabela, Jensen (6), working at the Federal Experiment Station at Mayagüez, undertook a study of the disease. He was not able to transmit it either mechanically or by grafting, but obtained evidence through field experiment that it was probably insect transmitted. As to the insects concerned, he did some preliminary experiments but without definite results.

For the past two years we have been engaged in the reinvestigation of papaya mosaic in Puerto Rico. As a result, three severe diseases, presumed to be caused by viral infection, have been identified among the papayas of the Island. The commonest of these is typical *bunchy top* disease, involving stunting;

[1] Published (June 1946) as Technical Paper No. 1 of the Agricultural Experiment Station, Rio Piedras, P. R.

[2] Plant Pathologist, Agricultural Experiment Station, University of Puerto Rico.

failure of latex flow on wounding; slow growth of short, stiff, nearly horizontal petioles; supporting small, thick, chlorotic leaves. A less common type of disease sometimes called *die-back* is distinguished by a severe chlorosis and defoliation from just below the growing point, progressing downward, and usually followed by necrosis of the tip of the stem or of the whole plant. In a small area of the Island including Guánica and Ponce, a typical *mosaic* disease seemed to replace the other types. This

FIG. 1—Left to right, leaves from diseased plants, showing deformation of leaf lamina. Extreme right, healthy leaf.

mosaic is characterized by mottling and extreme distortion of leaves, and also by the presence of green and brown rings on the fruits. It is still a question whether this mottling disease is closely related to typical *bunchy-top* and *die-back*, but the three conditions have some features in common. All three types of diseases are characterized by a marked reduction of top growth and especially by the sudden appearance of green streaks on the stem and young petioles. *Bunchy-top* and *die-back* are almost always characterized by failure of latex flow on wounding affected tissues, whether of leaf, fruit or stem; but this phenomenon is much less common in the mosaic type of disease. Our efforts to transmit either bunchy-top or die-back by mechanical means have so far proved fruitless, but we have been more successful in handling the mosaic type.

This paper deals with the transmission of papaya mosaic, resembling the so called papaya mosaic type "B", reported by Acuña and De Zayas (1) from Cuba.

FIG. 2.—Left, diseased plant showing stuntiness and leaf deformation. Markers show filiform deformity of lamina. Right, healthy control.

MATERIALS AND METHODS

The virus material used in the different experiments came originally from a diseased papaya plant grafted with tissue from a mosaic infected plant brought to the laboratories from the Ponce-Guánica region.

All transmission experiments were conducted on healthy papaya plants grown in 6 inch pots in the greenhouse, ranging from $1\frac{1}{2}$ to 3 months of age. Plants were taken to the laboratory and inoculated, using the pin-puncture method of Sein's (9), rubbing with carborundum, and grafting. In the pin-puncture technic, pieces of leaves, from infected plants showing early symptoms of the disease, were wrapped as closely as possible to the growing point and also about the middle of the stem and punctured repeatedly 1 to 15 times with an insect

needle. When the abrasive was used, an infected leaf was macerated in a mortar with a little sterile sand and water, the extracted juices were strained through cloth and the filtered

FIG. 3—Two plants showing atrophy of growth and leaf deformation.

juice applied with a cotton swab impregnated in the liquid, over one or two of the youngest leaves previously powdered with a little carborundum. Leaves were held in position with a clean wooden label.

In grafting, slips made with a razor blade from stems of infected plants showing oil streaks, were inserted into slits in the stem of the healthy stock, or slices from the cortical layers of healthy stem were taken and the infected slips firmly attached to it. In both cases, the grafts were covered with a piece of moist cotton and lightly tied with strings. The cotton was kept moist during the first few days by spraying with water from an atomizer.

All inoculated plants were kept in the laboratory from 4 to 5 days, then replanted into 10 inch. pots and taken to a wire screen shelter for further observations. Temperature inside

the shelter during the course of the experiment ranged from 74 to 90°F.

Controls were treated in the same manner, using materials from healthy plants for the different operations.

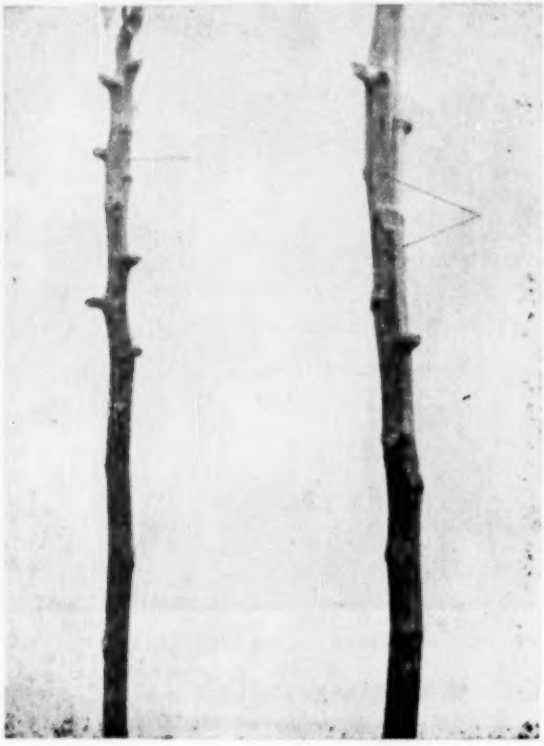


FIG. 4—Oily spots on stem of diseased plants.

TRANSMISSION

By pin punctures: Papaya mosaic was readily transmissible from diseased to healthy papayas by the use of a needle as described above. Eleven out of twenty-nine trees were infected by using this technic.

It was observed that, in general, the chances for a successful transmission were better if the leaves used as a source of inoculum were taken from the infected plant during the first two or three days after inception of the disease. Extracts made from leaves just beginning to show symptoms yielded a very infectious liquid.

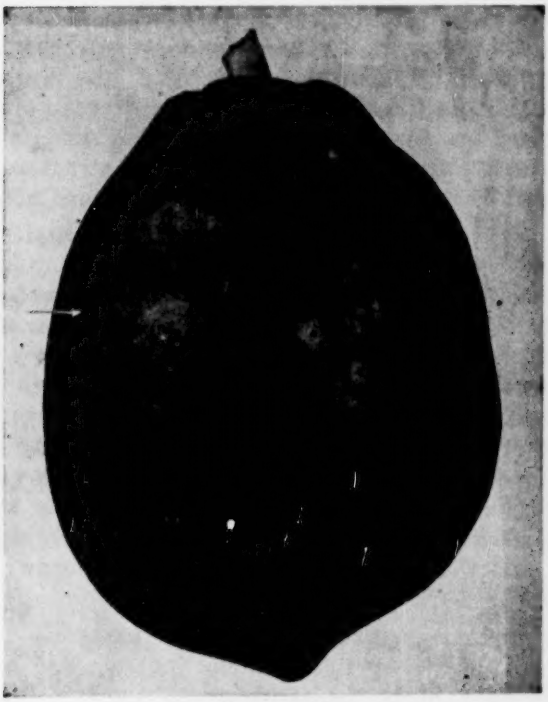


FIG. 5—Fruit taken from a diseased tree. Notice sharp rings on the surface of the fruit.

By the use of carborundum: Transmission by means of an abrasive (carborundum) was also readily accomplished. We have found this method to be a much easier and more effective way of transmitting the disease than pin puncture. Positive results were obtained in twenty three out of twenty eight inoculations.

By grafting: Pieces of stem tissues from infected plants grafted on healthy plants, in the manner described under Methods, transmitted the disease.

SYMPTOMS

Plants inoculated either by pin puncture or carborundum developed symptoms in 8 to 15 days after inoculation. Grafted plants took from two to three weeks before showing any evidence of the disease.

Inoculated and grafted plants exhibited at first a slight chlorosis of top leaves, followed by mottling, wrinkling and puckering. A light yellowing of the veins was also noticeable at this stage. As the disease progressed there was a marked deformation (figs. 1-2-3) and reduction of the leaf lamina, which in the majority of cases acquired a filiform structure. Leaves fully formed before onset of the disease remained normal.

A few days after the appearance of the initial symptoms, more or less elongated streaks, dark green in color (fig. 4), began to form about the middle of the stem, progressing upward and finally covering the whole length of the shoot. In only a few plants were we able to notice the occurrence of the streaks simultaneously with the appearance of primary symptoms. Concomitant with all these manifestations there was a gradual inhibition of the apical growth ultimately leaving the plant badly stunted (figs. 2 and 3), with a few small short petioles and distorted leaves on top. Two other distinguishing features of this disease were the flow of latex on wounding any part of the plant and the presence of green and dark-brown rings on the fruits (fig. 5).

The symptoms described above are generally present in field-affected plants, but the distortion of the leaves and atrophy of growth are not as severe as under laboratory conditions. Whether this is due to the influence of the environment on the expression of the disease, the age of the plant when inoculated, or other factors, we cannot say at present.

Discussion

Proper consideration of the published works on the so-called "Papaya Mosaic" diseases show that, although all of them present certain symptoms in common with the mosaic described in this paper, they, nevertheless, vary in certain other respects.

The diseases described from Trinidad (2), Hawaii (8), Cuba (Mosaic type "A") (1) and Puerto Rico (6), share with our mosaic the presence of oily spots on the stem and petioles; and the marked stunting of growth with reduction in the size of the leaves, internodes and petioles. They differ, however, in not showing the characteristic mottling and extreme distortion of the leaf lamina, which is a prominent feature of our disease. The Trinidad mosaic is further differentiated by the

fact that, according to Baker, it is characterized by "a rapid die-back starting from the crown a few weeks after infection." On the other hand, the Trinidad, Hawaii, Puerto Rico and Cuba diseases present some symptoms in common, but the first is again differentiated from the last three by the rapid die-back of the crown.

There is, however, another disease reported from Cuba as mosaic of papaya (type "B") (1), which insofar as can be judged from its description, seems to be related, if not identical, with the mosaic under consideration. It is pertinent to state that in our opinion, the mosaic (type "B") from Cuba closely fits into the description we have just given of our own mosaic. Added weight for this assumption is supplied by a photograph of a distorted leaf, included in the paper just mentioned, which is indistinguishable from our own material. Unfortunately, the Cuban investigators were unable to transmit the disease mechanically, so that definite comparisons of both diseases under controlled laboratory conditions are still wanting.

Summary

A mosaic disease of papaya is reported; characterized by stunting, with accompanying reduction in size of internodes, leaves and petioles, oily spots on stem and petioles, marked mottling and distortion of leaves, uninterrupted latex flow, and dark-green to brown rings on fruits.

The disease is transmitted by pin puncture, rubbing with carborundum and stem grafting.

The similarities and differences in symptomatology between this disease and others of similar nature reported from other parts of the world, are presented.

The disease is, however, believed to be closely related to, if not identical with, the mosaic type "B" from Cuba.

Resumen

Se ha hecho relación de una enfermedad denominada "mosaico" de la papaya, la cual se caracteriza por un detenimiento en el desarrollo de la planta, acompañado de reducción en el tamaño de los internodios, hojas y pecíolos; manchas oleaginosas en los tallos y pecíolos; marcado moteado y torcimiento de las hojas; interrupción del flujo del látex y círculos verdinegros o color castaño en las frutas.

La enfermedad se transmite mediante punturas con agujas, frotaciones con carborundo e injertos en los tallos.

Se han dado a conocer las similitudes y diferencias en la sintomatología entre esta enfermedad y otras de naturaleza parecida, sobre las cuales se han recibido informes de otros sitios del mundo.

La enfermedad está, por lo tanto, de acuerdo con el criterio del autor, íntimamente relacionada con el "mosaico" tipo "B" procedente de Cuba.

ACKNOWLEDGMENT

The author is deeply indebted to Dr. Francis O. Holmes, of The Rockefeller Institute for Medical Research at Princeton, without whose personal interest and unfailing advice this work would not have been accomplished.

LITERATURE CITED

1. **Acuña, J.** and **F. Zayas.** "Fruta bomba o papaya." Revista de Agr. (Cuba) 23: 40–3. 1939.
2. **Baker, R. E. D.** Papaw mosaic disease. Tropical Agri. (Trinidad) 16: 159–163. 1939.
3. **Cook, M. T.** New virus diseases of plants in Puerto Rico. Puerto Rico Dept. Agr. Jour. 15: 193–195. 1931.
4. **Cook, M. T.** New virus diseases in Puerto Rico. Phytopath 21: 124. 1931.
5. **Ho, W. T. H.** and **L. Y. Li.** Preliminary notes on the virus diseases of some economic plants in Kwang-tung Province. Lingnam Sci. Jour. 15(1): 67–78. 1936.
6. **Jensen, J. H.** Puerto Rico (Mayagüez) Exp. Sta. Ann. Report, 1937–38.
7. **Morwood, R. B.** Queensland Dept. Agr. and Stock Ann. Report., p. 47. 1931.
8. **Parris, G. K.** A new disease of papaya in Hawaii. Amer. Soc. Hort. Sci. 36: 263–65. 1938.
9. **Sein, F.** A new mechanical method for artificially transmitting sugar cane mosaic. Puerto Rico Dept. Agr. Jour. 14: 49–68. 1930.
10. **Simmonds, J. H.** Plant diseases and their control. Queensland Agr. and Past. Handbook Vol. 3. Brisbane. 1938.
11. **Smith, F. E. V.** Jamaica Dept. Agr. Ann. Rept., p. 18. 1931.
12. **Smith, F. E. V.** Jamaica Dept. Agr. Rept., p. 20. 1932.
13. **Smith, F. E. V.** Jamaica Dept. Agr. Ann. Rept., p. 19. 1939.
14. **Su, M. T.** Report of the Mycologist, Mandalay, Burma. Burma Dept. Agr. Rept. 1933–34, p. 25–33. 1934.

STUDIES ON VIRUS DISEASES OF PAPAYA (CARICA PAPAYA) IN PUERTO RICO [1]

II. Transmission of Papaya Mosaic by Green Citrus Aphid, (Aphis spiraecola Patch)

By JOSÉ ADSUAR [2]

As described in a recent paper (Adsuar 1945) a mosaic of papaya has been found attacking this crop in the southern coast of Puerto Rico. Although the disease has been transmitted both mechanically and by grafting, it was not until very recently that we have also successfully accomplished its transmission by means of the green citrus aphid, (*Aphis spiraecola* Patch).

This aphid, a major pest of citrus trees, in which it causes the well known "rosetting" of the leaves, seems to migrate to papayas during certain seasons of the year, at least under our tropical conditions. Originally described from material collected on spiraea, in Maine, it was first found in Puerto Rico on grapefruit in 1926. It was the fortunate coincidence of noting its presence on papayas during a routine inspection of our experimental plots, later confirmed for different sections of the island, including the southern coast, which led us to test its vector potentialities. This paper reports the experimental transmission of papaya mosaic by the green citrus aphid (*A. spiraecola* Patch).

TRANSMISSION STUDIES

Aphids, both nymphs or winged forms, were used in the different experiments. They were fed for periods varying from 8 min. to 1 hour on mottled leaves from papaya plants showing early symptoms of the disease and then transferred by means of a camel's hair brush to healthy plants about 3 months old caged in cellulose tube casings (Fig. 1). The number of aphids used for each transmission experiment varied from 6 to 10, but was never less than 6.

Controls were prepared by transferring aphids, handled in the same way, directly from citrus to healthy papaya plants.

[1] Published (June 1946) as Technical Paper No. 2 of the Agricultural Experiment Station, Rio Piedras, P. R.

[2] Plant Pathologist, Agricultural Experiment Station, University of Puerto Rico.

Aphids collected from experimental plants showing definite symptoms of the disease were sent to Dr. P. W. Mason at Washington, who identified them as *A. spiraecola* Patch.

Results

Positive transmissions were obtained in 15 out of 33 plants subjected to the action of *A. spiraecola Patch* fed on diseased papaya leaves for periods of time varying from 8 min. to 1 hour. Both nymphs and winged adults transmitted the disease with equal ease. All controls remained healthy.

The evidence seems to indicate that the aphids are avirulent while on the citrus leaves and that if they are instrumental in the propagation of the disease in the field, they must acquire the virus later during their migration. Further work is still needed to elucidate, among other things, whether *A. spiraecola* Patch is the only vector involved, when and where it become infectious, modes and seasons of migration to papaya plantations, retention of virus by the insect, etc.

Resultados

Se ha verificado la transmisión del mosaico o moteado de la papaya en Puerto Rico, a través del áfido conocido por pulgón cítrico verde, *A. spiraecola* Patch.

Este áfido es el causante de la enfermedad denominada "encaracolado" de las hojas de los cítricos.

Se obtuvieron transmisiones positivas en 15 de 33 plantas sometidas a la acción del áfido *A. spiraecola* Patch que se alimentó en hojas de papayos enfermos durante períodos de tiempo que variaron de 8 minutos a una hora. Tanto las ninfas como los adultos alados transmitieron la enfermedad con igual facilidad. Todos los testigos sometidos a la acción de los áfidos alimentados en hojas sanas permanecieron libres de la enfermedad.

La evidencia parece indicar que los áfidos adquieren el virus durante el proceso de emigración hacia los papayos. Se hace necesario todavía continuar los experimentos para dejar claramente sentado, entre otras cosas, si el áfido *A. spiraecola* Patch es el único vector envuelto en la transmisión de la enfermedad, cuándo y dónde llega a ser infeccioso, el modo y la época de emigración a las plantaciones de papayos, la retención del virus por el insecto, etc.

ACKNOWLEDGMENT

The writer wishes to make grateful acknowledgment to Dr. G. N. Wolcott, Head of our Department of Entomology, for information and advice on this aphid and for securing the specific identification through Dr. P. W. Mason, Specialist in the Bureau of Entomology, U.S.D.A.

LITERATURE CITED

Adsuar, J. Studies on Virus Diseases of Papaya (Carica Papaya) in Puerto Rico. I—Transmission of Papaya Mosaic. Agr. Exp. Station, Tech. Paper No. 1, pp. 1–8, 1946.

FIG. 1.—Papaya plant illustrating cellulose tube used in the experiments.

STUDIES ON VIRUS DISEASES OF PAPAYA (CARICA PAPAYA) IN PUERTO RICO

III. Property Studies of Papaya Mosaic Virus

By JOSÉ ADSUAR[1]

During the last two years a couple of virus diseases (Adsuar, 1, 2) have been found attacking papaya *Carica papaya* in Puerto Rico. One of them, the so called "bunchy top" disease, is of general occurrence through the island and resembles in general appearance the papaya viroses reported from Trinidad, Cuba and Santo Domingo. "Bunchy top" is extremely difficult, if not impossible, to transmit either mechanically or by grafting, and it has been only recently that we have been able to obtain evidence of its transmission by means of a leafhopper vector (3). The other disease, a typical *mosaic*, differs from "bunchy top" not only symptomatologically, but also in the fact that it is easily transmissible by mechanical means as well as by grafting. Its insect vector is an aphid (2).

Due to its stability *in vitro* and to the ease of mechanical transmission, experiments were undertaken to determine the physical properties, filterability, etc., of the papaya mosaic virus.

MATERIALS AND METHODS

The virus was obtained from artificially infected papaya plants kept inside wire-screened shelters. The plants were inoculated with the aid of carborundum following the method described by Adsuar (1). All experiments were conducted in sets of four plants each. After treatment the plants were transplanted into 12 inch pots and transferred to the shelters. The aphids used in the retention experiment were taken from papaya trees, starved, and then fed on infected leaves for the required periods of time, inside Petri dishes. After feeding was completed, they were removed and placed on healthy plants for the retention studies.

[1] Plant Pathologist, Agricultural Experiment Station, University of Puerto Rico.

Experimental Studies on the Papaya Mosaic Virus

Resistance to Heat:

Leaves from recent cases of *mosaic* were pounded in a mortar with sterile sand and 1 cc. of distilled water gradually added. The juice was expressed through heavy muslin, and about 2 cc. of juice obtained. To these more water was added, making a final dilution of approximately 1:12. Samples were then heated in corked tubes holding about 1¼ cc. each, to temperatures of 50°C, 55°C and 60°C for 3, 10 and 30 minutes periods at each temperature. (Table I).

Table No. 1

THERMAL INACTIVATION OF PAPAYA MOSAIC

Temperature °C	Minutes heated	Number of Plants Inoc	Infected
50	3	4	4
50	10	4	4
50	30	4	1
55	3	4	4
55	10	4	1
55	30	4	0
60	3	4	0
60	10	4	0
60	30	4	0

The results indicated that papaya mosaic virus has a thermal inactivation range between 55°C to 60°C for 10 min. Exposures to 55°C for 10 min. show a decrease of infectivity, with complete inactivation at 60°C for the same period of time.

Tolerance to Dilution:

Infected leaves showing early symptoms were crushed in a mortar with a little sterile sand and distilled water, the pulp squeezed through muslin, and the resulting green fluid diluted as follows: 1:4; 1:16; 1:64; 1:256 and 1:1024.

The virus remained infective up to and including 1:256. No infections were obtained with the 1:1024 dilution.

Longevity in vitro at room temperature:

Samples obtained as above and diluted approximately 1:12 were stored at laboratory temperature (29°C) and tested at 24, 48 and 72 hrs. No fermentation or putrefaction was noted. Chlorophyll settled leaving a clear fluid above. Part of the same juice was put in the refrigerator at 10°C and tested at different intervals of time.

The results indicated that the virus is completely inactivated in 48 hrs. at room temperature. At refrigerator temperature, the virus was still active at the end of 15 days.

Filterability:

Leaves of plants showing the disease were ground with approximately three times their weight of distilled water. Juice was extracted through muslin and centrifuged for 15 minutes in a clinical centrifuge. To the supernatant fluid was added ¼ cc. of a 19 hr. culture of *Sarcina lutea* in broth (Difco). The mixture was then filtered through a Seitz disc No. 5114-C10.

The filtrate proved to be free from bacteria as determined by inoculation in sterile nutrient broth and on nutrient agar slants. The virus passed the filter, but apparently did so with much difficulty. Of our plants inoculated, only one came down with symptoms. Controls inoculated with the unfiltered mixture all showed the disease.

Inactivation by Drying:

Recently affected young leaves (showing vein clearing and chlorotic spotting) from previously inoculated plants were picked, tied together and suspended in a shady but breezy part of the laboratory for drying. Some of the leaves were macerated immediately after collection with the addition of 2 cc. of water and the juice tested for infectivity. The rest of the leaves were allowed to dry for 48 hours and 72 hours periods. Samples extracted as explained above were obtained at the required time and inoculated on healthy plants. Plants inoculated with juice from leaves extracted immediately after picking all came down with the disease. None of the plants inoculated with juice extracted from leaves air dried from 48 to 72 hours became infected.

Retention of Virus by Aphids:

Winged adults of *Aphis spiraecola* Patch were collected and starved for about 1 hour, transfered to infected leaves, and allowed to feed for 15 minutes. Aphis in lots of five were then taken to individual papaya seedling and allowed to feed for 3 hours. After that interval they were removed to new seedlings. Table 2 shows the result of the experiment.

TABLE No. 2

RETENTION OF PAPAYA MOSAIC BY APHIS SPIRAECOLA PATCH

Lot	Number Aphids Used	Plants inoculated during 3 hrs. period	Plants infected during 3 hrs. period	Plants inoculated after 3 hrs. period	Plants infected after 3 hrs. period
1	5	1	1	1	0
2	5	1	1	1	0
3	5	1	1	1	0
4	5	1	0	1	0

The results demonstrated that the aphids failed to retain the virus for more than 3 hours.

SUMMARY

The physical properties of papaya mosaic virus are reported. The virus is inactivated by a 10-minute exposure at 60°C. No infection was obtained when juice was diluted up to 1:1000. The virus was completely inactivated in 48 hours at room temperature. Filterability was accomplished but with difficulty through a Seitz germicide filter. The virus was found to lose its infectivity in the leaves when air dried for 48 hours.

Aphis spiraecola Patch retained the virus the first 3 hours but failed to infect a second lot of plants after that time.

RESUMEN

Se ha informado sobre las propiedades físicas del virus del mosaico de la papaya. El virus queda inactivo, mediante una exposición a 60°C durante 10 minutos. No se obtuvo infección alguna cuando el jugo fué diluído hasta 1:1000. El virus queda competamente inactivo en el término de 48 horas a la temperatura del laboratorio. Se efectuó con dificultad la filtración a través de un filtro tipo Seitz No. 5114-C10. Se descubrió que el virus perdió su infectividad en las hojas expuestas al aire durante 48 horas.

El afídido *A. spiraecola* Patch retuvo el virus durante las primeras tres horas; pero, después de ese tiempo, no logró infectar un segundo número de plantas.

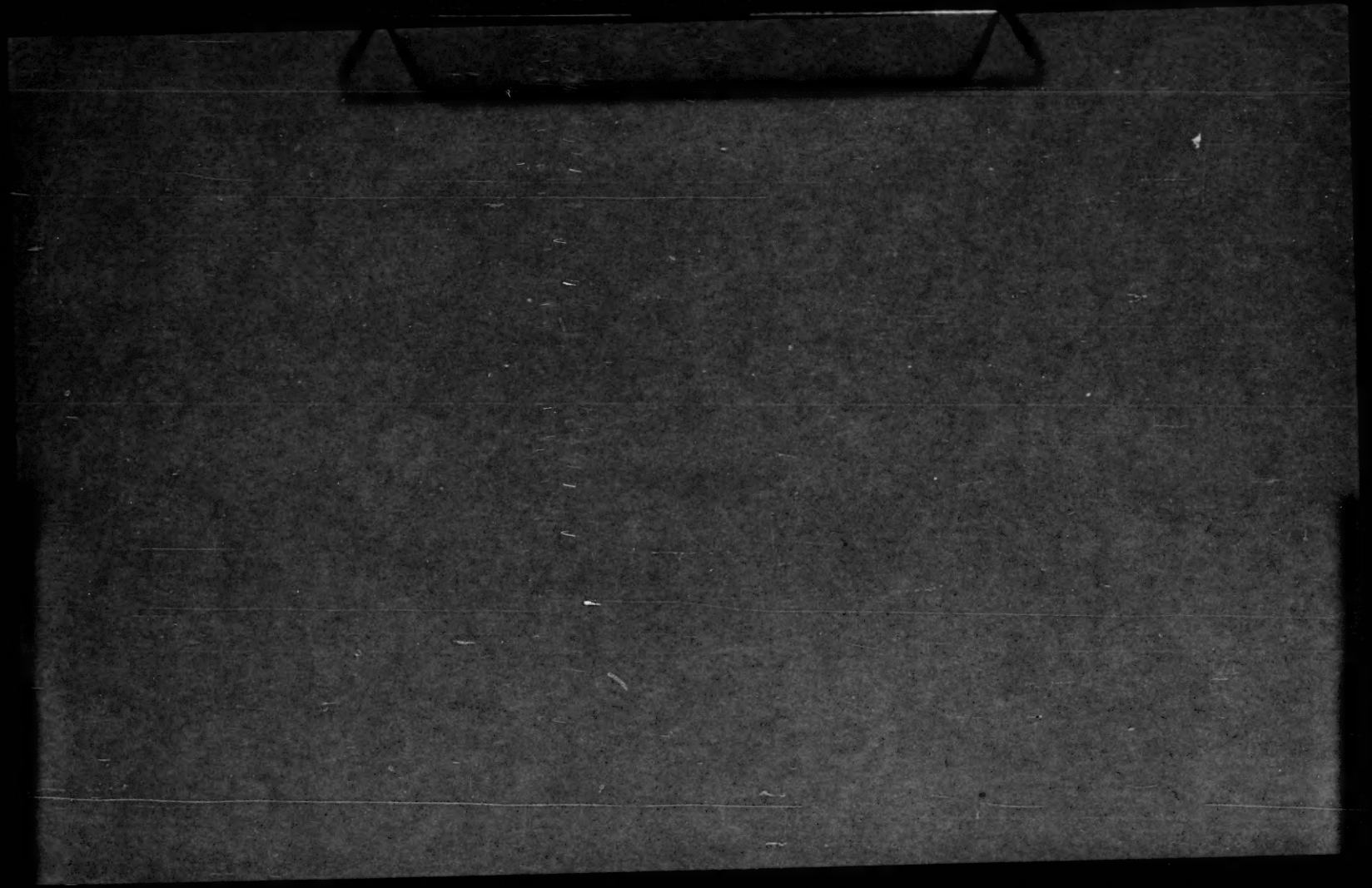
ACKNOWLEDGMENT

The writer again wishes to acknowledge his gratitude to Dr. Francis O. Holmes of the Rockefeller Institute at Princeton, New Jersey, for his disinterested and helpful collaboration in carrying on this work.

REFERENCES

1. **Adsuar, J.** Studies of Virus Diseases of Papaya (Carica papaya) in Puerto Rico. I—Transmission of Papaya Mosaic. University of P. R., Agricultural Experiment Station, Río Piedras. Tech. Paper **1**: 1–9. 1946.

2. ---------- Studies on Virus Diseases of Papaya (Carica papaya), in Puerto Rico. II—Transmission of Papaya Mosaic by the Green Citrus Aphid (**A. spiraecola** Patch.) University of Puerto Rico, Río Piedras. Tech. Paper **2**: 1–3. 1946.

3. ---------- Transmission of Papaya Bunchy-top by a leafhopper of the Genus Empoasca. Science **103** No. 2671: 316. 1946.

0—0—0